555 and 647) positioned on each side of both operators allow detection of all possible looping topologies (two parallel and two antiparallel trajectories). Comparisons among these fluorophore position variants allow measurement of the population of each loop state. Finally, we have studied the effect of IPTG on loop geometry and stability. Bulk and single-molecule FRET studies show that: (1) Remarkably, all the constructs on the landscape form stable loops. (2) Parallel and antiparallel topologies have different LacI geometries detected by different FRET efficiencies, probably due to differential effects of DNA stiffness in the two contexts. (3) Some constructs form an extended loop that can be identified by loss of FRET. (4) Induction affords a mixture of the initial loop, a state with decreased FRET, and additional FRET-silent states that we tentatively ascribe to specific-nonspecific loops. (5) States on the borders of the landscape regions comprising uniform loops are maximally sensitive to IPTG. The overall results do not agree with rod mechanics models that consider only DNA bending and flexibility, confirming that protein flexibility must be considered in modeling DNA loops.

375-Pos Board B175
Kinetic Studies of Lambda Repressor-Mediated DNA Looping Suggest Physiological Role for Non-Specific Binding
Laura Finzi, Carlo Manzo, Chiara Zurla, Sankar Adhya, David D. Dunlap.
The kinetics of DNA loop formation and breakdown by the λ repressor or CI protein was characterized using the tether particle motion technique (TPM) and a novel method of analysis with increased time resolution. The kinetics of loop formation was described by a stretch exponential, while the kinetics of loop breakdown was found to be more complex and only the distribution of the long dwell times could be described by a power law. Comparison with the kinetics of loop formation and breakdown induced in DNA with mutated operators, shows that repressor bound at these sites may act as a nucleation site for further binding and loop stabilization, and may contribute to broaden the rate constants distribution. A model is suggested by which nonspecific binding of CI along the inter-operator distance may shorten the effective separation between the specific sites which mediate looping thereby lowering the potential energy necessary for loop formation. Finally, CI-mediated looping kinetics also shows that the frequency of transition between the looped and unlooped DNA conformation does not vary with CI concentration, despite the fact that the loop becomes thermodynamically more stable. The relevance of this feature to the robustness of the system is discussed.

376-Pos Board B176
The Role of Architectural Proteins in Lac-Mediated DNA Looping
Luke Czapla, David Swigon, Wilma K. Olson.
The widely abundant nucleoid protein HU contributes to both the spatial organization and biological processing of bacterial DNA. One such contribution of HU to biological processing is tied to gene regulation mediated by the looping of DNA induced by proteins, such as the tetrameric Lac Repressor protein, which simultaneously binds two distant operator DNA sites in the bacterial genome. Current understanding of how HU might contribute to the transcription of the *lac* genes derives from indirect theoretical and computational analysis of the effects of chain length on gene expression in *E. coli* cells with and without HU. We take a more direct approach to the question of how architectural proteins mediate the structure and looping of DNA *in vivo*, incorporating the structural effects of both HU and Lac Repressor on DNA in Monte Carlo simulations, taking advantage of new methods that we have developed to understand the properties of protein-bound DNA. We present our predictions from models for understanding Lac-mediated gene expression *in vivo,* and also demonstrate state-of-the-art calculations that incorporate our structure-based models in order to obtain more detailed insights into the phenomena observed in ring-closure experiments of DNA in the presence of architectural proteins.

377-Pos Board B177
Divide a Plasmid DNA Molecule into Two Independent Superhelical Domains by Sequence-Specific DNA-Binding Proteins: A DNA Superhelical Barrier Model
Fenfei Leng, Bo Chen.
Both prokaryotic and eukaryotic chromosome are organized into many independent topological domains. These topological domains are presumably formed through constraining each DNA end from rotating by the interaction with nuclear proteins, *i.e.,* DNA-binding proteins. However, so far, there is no direct evidence to support this hypothesis. In this study, we utilized two new *in vitro* methods, developed in our laboratory to examine whether certain sequence-specific DNA-binding proteins can separate a plasmid DNA molecule into different DNA superhelical domains. Our new methods are based on the successful construction of several plasmid DNA templates that contain many tandem copies of one DNA-binding sites in two different locations (, B., Xiao, Y., Liu, C., Li, C., and Leng, F. (2010) *Nucleic Acids Resesearch,* 38, 3643-3654). Using these new methods we discovered that several sequence-specific DNA-binding proteins, *i.e.,* LacI, GalR, AraC, λ O protein, can divide a plasmid DNA molecule into two independent superhelical domains. These independent superhelical domains are thermodynamically stable. Interestingly, CRP (E. coli cAMP receptor protein), a DNA-bind and -bending protein, cannot divide the plasmid DNA molecule into different DNA topological domains. Our results can be explained by a superhelical barrier model of nucleoprotein complexes in which DNA supercoils may be confined in localized regions. We propose that the DNA superhelical barriers are certain nucleoprotein complexes that contain stable toroidal supercoils assembled from DNA looping or tightly wrapping DNA around DNA-binding proteins. The biological significance of the new superhelical barrier model will be discussed. This work is supported by a NIH grant 5SC1HD063059-02.

378-Pos Board B178
Single-Molecule Observation of Dynamic Bending and Cleavage of a Gate DNA by Human Topoisomerase IIα
Sanghwa Lee, Seung-Ryoung Jung, Joseph E. Deweese, Jo Ann Byl, Neil Osheroff, Sungchul Hohng.
Type II topoisomerases are an essential enzyme that resolves intrinsic topological problems encountered during DNA replication and RNA transcription. They have been a major target of antibacterial and anticancer drugs. Decades of research established that these ATP-dependent molecular machines operate by transporting a DNA duplex (the transport or T-segment) through a transient break in another DNA duplex (the gate or G-segment). However, mechanistic steps of the enzyme's catalytic cycle and their dynamics remain largely uncharacterized, and it is still an intriguing question how the complex series of conformational changes in DNA substrates and the enzyme are communicated, and integrated into a coordinated overall reaction cycle. Here we describe single-molecule fluorescence experiments to monitor the association/dissociation dynamics of human topoisomerase IIα, and accompanying bending/cleavage events of G-segment DNA. Our observation reveals that 1) In the presence of divalent ions, dynamic bending of G-segment, an intermediate step to the cleavage reaction, occurs in cleavage-competent sequences, and 2) The sequence specificity and efficiency of the cleavage reaction is determined by the deformability of the sequence, rather than by the chemical information stored in the sequence. 3) DNA cleavage reaction, which is tightly down-regulated to a minimum level, is greatly accelerated by the clamping motion of N-gate induced by nucleotide binding.

379-Pos Board B179
Single Molecule Rupture Force Measurements of TOPOII-DNA Binding
Yii-Lih Lin, Yi-Ren Chang, Tzu-Ming Ou, Chia-Shen Chang, Ting-Fang Wang, **Chia-Fu Chou**.
Using AFM and magnetic tweezers, we performed single molecule rupture force measurements on the binding of Topoisomerase (TOPO) II to double-stranded DNA. With a few hundred pulling events, using DNA and disulfide bond cross-linked mutant TOPOII as controls, we found the rupture force between wild type TOPOII-DNA complex is ~45 pN at a 50% accumulative probability.

380-Pos Board B180
Investigating the Interaction of Single UvrA Dimers with DNA Using a Combination of Fluorescent Microscopy and Optical Tweezers
Andreas Biebricher, Koen Wagner, Geri F. Moolenaar, Nora Goosen, Remus T. Dame, Erwin J.G. Peterman, Gijs J.L. Wuite.
Nucleotide excision repair (NER) is a DNA repair mechanism responsible for replacement of base pairs damaged e.g. by exposure to UV light. In *E. coli*, NER is initiated by the UvrA-dimer, which searches for and localizes damaged nucleotides to which then the UvrB-dimer is recruited. We study the damage search of UvrA by visualizing single, fluorescently labeled UvrA-dimers interacting with a DNA molecule manipulated by double optical tweezers. This approach enables us to observe UvrA binding in real time to DNA under different tensions.
We find that, in the presence of ATP, the UvrA-dimer displays static, non-specific DNA binding of long duration (>10s), in accord with a recent publication [1]. In addition, our results reveal that non-specific UvrA binding is tension dependent: Binding events are prolonged on DNA under tension, indicating that the dissociation rate decreases while the association rate remains rather constant, thus supporting a model in which UvrA binding unwinds DNA [2].
Finally, we observe that the duration of binding events decreases by several orders of magnitude if ADP instead of ATP is present in the buffer (<<1s). Binding is sensitive to tension, even more so than in the ATP experiment, resulting in a more than tenfold increase of the duration of binding events when DNA tension is increased from 5 to 65pN. In addition, our results demonstrate that UvrA has a higher binding affinity for ADP than for ATP. We are currently investigating whether ADP binding plays an important role in accelerating the DNA damage search *in vivo*.
[1] Kad, N. M., Wang, H., Kennedey, G. G., Warshaw, D. M., van Houten, B. (2010). Mol. Cell *37*, 702–713.
[2] Oh, E., Grossman, L. (1986). Nucleic Acids Res. *14*, 8557–8571.